

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 19, lines 20-26 with the following amended paragraph:

Figure 6 shows how an *Xba*I site can be made sensitive to methylation.

The RE cuts at the sites shown by arrows. The boxed sequence is methylated in a *dam*⁺ strain thereby altering the *Xba*I recognition site. The sequence however is not methylated in a *dam*⁻ strain, and so can still be cleaved by *Xba*I. The *Xba*I recognition sequence (5'TCTAGA3') can therefore be selectively cleaved by *Xba*I I. Assembly of DNA units uses only one restriction enzyme - *Xba*I. Figure 6 discloses SEQ ID NOS:1-2, respectively, in order of appearance.

Please replace the paragraph beginning at page 20, lines 19-24 with the following amended paragraph:

Figure 13 shows the amino acid sequence alignment of the *recA* protein of *S. lividans* (S.l.) (SEQ ID NO:3) and *S. ambofaciens* (S.a.) (SEQ ID NO:4). Percent similarity: 96.496, percent identity: 95.418. Match display thresholds for the alignment(s):

I = identity

: = 2

. = 1

Please replace the paragraph beginning at page 20, lines 26-28 with the following amended paragraph:

Figures 14A and 14B show a DNA sequence alignment of the *recA* gene *S. lividans* (S.l.) SEQ ID NO:5) and *S. ambofaciens* (S.a.) SEQ ID NO:6). Start of the gene is from 'ATG' and stop is 'TGA'. Percent similarity: 94.713, percent identity: 94. 713.

Please replace the paragraph beginning at page 25, lines 9-16 with the following amended paragraph:

A similar system to the *XbaI/dam* system described above, uses the restriction enzyme *FokI* which has the recognition site:

5' GGATG(N)₉ 3' (SEQ ID NO:7)

3' CCTAC(N)₁₃ 5' (SEQ ID NO:8)

with the *dcm* methylase of *E. coli*. Adding CCA or CCT to the 5' end of the *FokI* recognition site would make the site *dcm* sensitive. Furthermore, if the sequence TCTAGA were inserted into the redundant section of the *FokI* restriction site, then the enzyme could be used to generate '*XbaI*-cut ends'.

Please replace the paragraph beginning at page 25, line 20, through page 27, lines 1-10 with the following amended paragraph:

Construction of the final expression plasmid pAR1 0 was carried out in several steps, as follows. The ten PKS DNA units were amplified by PCR using *pfu* DNA polymerase. The respective regions of *eryAI* gene, as well as the oligonucleotides used for each PCR are outlined:

LM - segment of *eryAI* gene (Bevitt *et al.*, 1992) extending from nucleotide (N) 588 to N 2389;

5'GGCATATGGCGGACCTGTCAAAGCTCTCCGACAGT3' (SEQ ID NO:9) and

5'GGTCTAGATCCCAGCCGCGGTTCGGTCGGCAGTCCCG3' (SEQ ID NO:10);

KS1 - segment of *eryAI* gene extending from N 2384 to N 3769;

5'GGTCTAGACTCGCTGTTCCACCCGACCCACGCGCTCGGGCACCGCGCACCA3'

(SEQ ID NO:11) and

5'GGTCTAGATCGCGCAGCGCGGCGGACTCGTCGACGGGGGCGAAGGCGG3' (SEQ ID NO:12),

AT1 - segment of *eryAI* gene extending from N 3764 to N 4813;

5'GGTCTAGACGGTCTCGCGACGGGAAACGCCGACGGTGCCGCCGTTGGAA3' (SEQ ID NO:13)

and

5'GGTCTAGATCCACCGCGACACCGGCGGGCGAACGCGCGGGAGAGCGCTTCGC3'

(SEQ ID NO:14),

KR1 - segment of *eryAI* gene extending from N 4808 to N 6316;

5'GGTCTAGAGTCGGTGCACCTGGGCACCGGAGCACGCCGGGTGCCCTT3' SEQ ID NO:15)

and

5'GGTCTAGATCGTCGAAGAGCCTGGTCGGGCGCTGCGCGGTGTA3' SEQ ID NO:16),

ACP 1 - segment of *eryAI* gene extending from N 6311 to N 6679;

5'GGTCTAGACGACGCGCGGGCTGCGCCGCAGGCGCCGGCCGAACCGCGGG3' (SEQ ID NO:17)

and

5'GGTCTAGATCGGCCGTGGTCGCCGGTGCCGCCTGCTCGGCT3' SEQ ID NO:18),

KS2 - segment of *eryAI* gene extending from N 6674 to N 8200;

5'GGTCTAGACGAGCCGATCGCGATCGTCGGCATGGCGTGCCGGCTGC3' (SEQ ID NO:19)

and

5'GGTCTAGATCGTGCACGGCCTCGGCGGTGTCGGCGGCGAGCACCGCGGCCCGCTC CTC3' (SEQ ID NO:20),

AT2 - segment of *eryAI* gene extending from N 8195 to N 9340;

5'GGTCTAGAGGCGGTGGCCGACGGCGCGGTGGTT3' (SEQ ID NO:21)

and

5'GGTCTAGATCGTCAC GAG G G GTG GTG CG GTCCG GCAG CAG CCAGAA3' (SEQ ID NO:22),

KR2 - segment of *eryAI* gene extending from N 9335 to N 10639;

5'GGTCTAGACGGCTGGTTCTACCGGGTCGACTGGACCGAG3' (SEQ ID NO:23)

and

5'GGTCTAGATCCGGCCGGGGCCGGGCGGCGGTGTAGGACT3' (SEQ ID NO:24),

ACP2 - segment of *eryAI* gene extending from N 10634 to N 10966;

5'GGTCTAGACCGCATCGTCACGACCGCGCCGAGCGA3' (SEQ ID NO:25) and

5'GGTCTAGATCGGCGTCGAGGAAA3' (SEQ ID NO:26).

TE - segment of *eryAIII* gene (Donadio *et al.* 1991) extending from N 8753 to N 9602;

5'GGTCTAGACAGCGGGACTCCCGCCCGGGAAGCG3' (SEQ ID NO:27) and

5'GGGCTAGCTCTAGATCATGAATTCCCTCCGCCAGCCAGGCGTC3' (SEQ ID NO:28).

Please replace the paragraph beginning at page 39, lines 1-20 with the following amended paragraph:

The oligonucleotides:

5'- GGTCTAGAATTCGGCAAGGGCGCCGGTCATGCGCAT-3' (SEQ ID NO29)

and 5'- GG TCTAGA TGTGCGGCGTCGGCCGGGGCGGCGGAGGCG-3' (SEQ ID NO:30)

were used as the forward and reverse primers respectively and the 1000 bp internal region of *S. lividans recA* gene (Nussbaumer and Wohlleben, 1994) was amplified using pfu polymerase. An additional nucleotide (B) was incorporated into the forward primer to generate a frame shift in the amplified *recA* gene fragment. The PCR product was cloned in pUC-18 vector and sequenced to detect for possible errors during PCR. The 1.0 kbp *recA* fragment, flanked at both ends by an *XbaI* site was then inserted in the expression vector pCJR24 that has a unique *XbaI* site. The ligation mixture was used to transform *E. coli* DH10B cells and the desired plasmid DNA isolated. The resulting plasmid (pARecA24) contains a non-methylated *XbaI* site at the 5' end of the *recA* gene fragment. The ten PKS DNA units, namely, TE, two each of ACP1, KR1, AT1 & KS1, and LM were inserted into the plasmid pARecA24 to finally yield the expression plasmid pRecAD1TE. This plasmid was used to transform wild-type *S. lividans* protoplasts, and thiostrepton resistant colonies were grown in defined liquid media as described above. The compound (Figure 12) was isolated from the bacterial broth and chemically characterised.